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RPPR Final Report
as of 09-Jan-2018

Agency Code:

Proposal Number: 68816LSREP **Agreement Number: W911NF-16-1-0424**
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Report Date: 14-Oct-2017 Date Received: 09-Jan-2018
Final Report for Period Beginning 15-Jul-2016 and Ending 14-Jul-2017
Title: Peptidomimetics as Chemical Probes of Protein-Protein Interactions
Begin Performance Period: 15-Jul-2016 **End Performance Period:** 14-Jul-2017
Report Term: 0-Other

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STEM Degrees: **STEM Participants:**

Major Goals: The overall goals of this project were to acquire new equipment for the purpose to improve the research capabilities of California State University, Fullerton (CSUF) and to strengthen student research abilities in scientific research in order to pursue STEM careers. This was accomplished by obtaining new equipment that was used for three research projects on the design and synthesis of peptidomimetics that will probe molecular interactions of proteins related to human heath and disease. These projects involved undergraduate students synthesizing novel molecules and studying their biological properties in additional to performing molecular modeling. The equipment that was purchased and installed for undergraduate student research: Liquid chromatography/mass spectrometer, combiflash purification system, rotary evaporators, a plate reader, high throughput reagent dispenser and computers with computational modeling software. Students have been trained and become confident users of the equipment for routinely use in their research projects. The new equipment has improved both the pace and quality of research results, which has increased the number of students presenting their research at scientific conferences. This has been an added benefit to the new equipment as student presentations at conferences is a great opportunity for students to gain support for pursuing a STEM career. The equipment has enriched undergraduate student research activities and helped promote careers in undergraduate research.

Accomplishments: The funded grant provided equipment for the synthesis and analysis of chemical probes to understand binding interactions between small molecules and proteins related to human health and disease. There

were three projects described in the funded grant that focused on synthesis and biological evaluation of small molecules.

Project 1. alpha-HELIX MIMETICS FOR INHIBITION OF PROTEIN-PROTEIN INTERACTIONS

In this project small molecules were proposed to be synthesized as alpha helix mimetics to probe the protein-protein interaction of the E6-E6AP complex. This complex is important for the human papillomavirus viral (HPV) survival in host cells. Understanding this protein-protein interaction will provide new information for the design of small molecules that can disrupt this complex ending HPV infection in host cells. A ten-step synthesis was proposed to create novel alpha-helix mimetics. The synthesis of the alpha-helix mimetics was not completed during the period of the grant. Several of the synthetic steps did not produced the expected products or give low yields that drastically slowed research progress. Through the course of the research the group did improve many of the chemical reactions in the proposed synthetic schemes in terms of reaction yields and are one synthetic step away from synthesizing the 1st set of small molecule probes. The major outcome from this work is the optimization of the synthetic route for the synthesis of alpha-mimetics. The research team is also investigating new alternative synthetic routes to create alpha-helix mimetics in a shorter number of synthetic steps.

Project. 2. Botulinum neurotoxin

The 2nd project is the synthesis and evaluation of small molecules that bind to the light chain of the botulinum neurotoxin (BoNT) and disrupt binding between the light chain and the protein substrate. The BoNT is the lethal agent that causes botulism, a disease that can result in muscle paralysis and death. The BoNT is composed of a heavy and light chain and acts in concert to cleave protein responsible for neurotransmission. The heavy chain binds to the cellular membrane and inserts the light chain in to the cytosol. The light chain is a zinc metalloprotease that cleaves SNARE proteins causing termination of neurotransmission resulting in paralysis. There is a fear the neurotoxin could be used for bioterrorism and investigation into small molecule light chain interactions are needed. The goal of the project was to synthesize novel small molecules and probe binding interactions through enzyme inhibition assays. Over the course of the grant a library of small molecules were synthesized and evaluated for inhibition of the light chain enzymatic activity as a method to probe binding interactions between small molecules and the light chain. Our initial objective was to synthesis molecules to probe the binding interactions by altering the substituents on the biphenyl ring. We completed this objective by synthesizing analogs and analyzed their ability to inhibit the protease. Our results indicated that the chlorine on the 3-position of the biphenyl ring resulted in the strongest interactions with the light chain. We believe this is due to the chlorine having a favorable interaction with an arginine residue in the active site.

In addition we synthesized additional analogs to probe two other important aspects of our scaffold, the sulfonyl amide bond and the amino acid core. We synthesized several other small molecule libraries and determined their ability to inhibit the light chain. Based on the analogs synthesized and evaluated in our assay, it was determined that an amide linker between the biphenyl ring and the amino acid had improved binding over the original sulfonyl amide. We believe the linker causes our molecule to adopt a specific molecular shape that encourages binding in the active site of the protease. The amide linker, which contains a carbonyl results in a more planar shape, while the sulfonyl amide assumes a more bent shape due to the oxygens binding to the sulfur of the sulfonyl amide. We are current performing docking studies to understand the binding versus shape dynamic of our small molecules interacting in the active site.

We also investigated the number of amino acids in our scaffold. Our initial scaffold contained one amino acid. Previously we determined that isoleucine was the preferred amino acid for binding to the BoNT protease. We incorporated an additional amino acid to observe how this affected binding to the protease. We screened hydrophobic amino acids since the active site of the protease is relatively hydrophobic with a large number of aromatic side chains exposed. This study led to phenylalanine and isoleucine as the optimal amino acids for our new scaffold. We then returned to studying the biphenyl ring structure with our new two amino acid core. It was hypothesized that the additional amino acid would drastically increase the overall lengthen of our inhibitor and would lead to poor binding in the active site. We compensated for this by replacing our biphenyl ring to a phenyl ring. We also studied the effects of chlorines attached to the phenyl ring as these halides were important for our initial inhibitor. This new scaffold has resulted in our best binders to date. These are strong binders with inhibitor concentrations that lower catalytic activity by 50% (IC50) in the nanomolar region.

Project 3.

The 3rd project described in the awarded grant is the synthesis of small molecules to probe binding to the active site of the West Nile virus (WNV) NS2B-NS3 protease. The WNV infects millions of people each year with a significant number of infected individual progressing into more severe diseases. We proposed to study small molecule protein binding interactions through a structure activity relationship study with zafirlukast, our initially discovered inhibitor for the NS2B-NS3 protease. Zafirlukast is a relatively large molecule with many functional groups that can be modified to study binding interactions. We synthesized all the proposed small molecule libraries

and examined their binding to the protease through an enzymatic inhibition assay. Our results indicated that several structural elements are needed for binding to the NS2B-NS3 protease active site. Building from the indole core we determined that the carbamate attached at the 5-position was required for binding to the active site. A further study of the carbamate reveled that a phenyl carbamate had improved inhibition over the orginal cyclopentyl group. This would indicate that aromatic residues are exposed in the active site and can used as binding partners for aromatic group on small molecules. In addition, the toluic amide component attached at the 3 position was also needed for binding. Interesting when the toluic amide was connected at the 1-N position of the indole, inhibition was similar to the analog at the 3 position. We believe that the length of the molecule is essential for binding and the two halves of the molecule need to be at a specific distance to make important binding contacts in the active site. We are currently analyzing this binding through computational docking analysis with the goal of designing new and better binders for the WNV NS2B-NS3 protease. In addition we are exploring new molecular linkers that could be incorporated into a scaffold that features the carbamate phenyl and toluic amide groups.
Overall these projects have increased student training and participation in scientific research specifically in the field of medicinal chemistry. The equipment obtained through this grant has lead to increased output of high quality research with a large number of student gaining valuable training and experience in drug design and synthesis.

Training Opportunities: All of the equipment obtained from this funded grant was installed and available to faculty and students at California State University Fullerton and neighboring high school students. Dr. Salzameda, one of the key personal listed on this funding award, trained faculty, graduate, undergraduate and high school students on the usage of the equipment. Training sessions were performed in one-on-one sessions or in small groups depending on the equipment. All training sessions covered the fundamentals of the equipment, routine operations and safety requirements. Once students and faculty were trained they were allowed to use the equipment freely. Many students have become expert users of the equipment and have integrated the equipment into their daily research activities. The knowledge and skills gained from using the equipment funded by this grant has enriched their research skills and will lead to high quality employment or enter graduate school research programs

Results Dissemination: The equipment funded by this grant has been used to collect scientific data that have been disseminated through several outlets. The major source of dissemination has been through undergraduate poster sessions. Students working in research laboratories in the Department of Chemistry and Biochemistry at CSUF are encouraged to present their research at scientific conferences. Students have presented their research using the equipment purchased with funds from this grant at a variety of conferences such as the American Chemical Society national meeting, California State University Program in Biotechnology Research and Education, Southern California Conference on Undergraduate Research and ACS Southern California Undergraduate Research Conference.

List of poster presentation by students:
Alejandro Torres, Nicholas T. Salzameda “Synthesis of Arginine Mimetics for the Inhibition of the West Nile Virus's NS2B-NS3 Protease” University of California, Los Angeles, American Chemical Society- Southern California Undergraduate Research Conference (ACS-SCURC), April 2017.

Giovanna Cano, Anastasia Martinez, Nicholas T. Salzameda “Synthesis of Peptidomimetics for Inhibition of NS2B/NS3 Protease” University of California, Los Angeles, American Chemical Society- Southern California Undergraduate Research Conference (ACS-SCURC), April 2017.

Martin Amezcua, Ricardo Cruz, Sandra Rodriguez Beltran, Nicholas T. Salzameda “Synthesis and Evaluation of Hydroxamic Acids against BoNT/A” University of California, Los Angeles, American Chemical Society- Southern California Undergraduate Research Conference (ACS-SCURC), April 2017.

Sandra L. Rodriguez Beltran, Thanh Lien, and Nicholas T. Salzameda “Structure activity relationship study of isoleucine sulfonamide hydroxamic acid inhibitors for the Botulinum Neurotoxin” San Francisco, American Chemical Society, April 2017.

Martin Amezcua, Ricardo Cruz, Sandra Rodriguez Beltran, Nicholas T. Salzameda “Synthesis and Evaluation of Hydroxamic Acids against Botulinum Neurotoxin” California State University, Fullerton, Chemistry and Biochemistry Departmental Poster Presentation, May 2017.

Alejandro Torres, Nicholas T. Salzameda “Synthesis of Arginine Mimetics for the Inhibition of the West Nile Virus's NS2B-NS3 Protease” California State Polytechnic University, Pomona, Southern California Conference for Undergraduate Research (SCCUR), November 2017.

Elizabeth Morales, Alejandra Y. Palomino, Nicholas T. Salzameda “Investigating Peptidomimetic Scaffolds for Inhibition of Botulinum Neurotoxin Light Chain” California State Polytechnic University, Pomona, Southern California Conference for Undergraduate Research (SCCUR), November 2017.

Elizabeth Morales, Alejandra Y. Palomino, Nicholas T. Salzameda “Investigating Peptidomimetic Scaffolds for Inhibition of Botulinum Neurotoxin Light Chain” Ensenada, International Symposium on Applied Research (ISAR), November 2017.

Trent Northen, Nicholas T. Salzameda “Discovery of a Small Molecule Inhibitors for the Cruz in Cysteine Protease in Chagas’ Disease” California State University, Fullerton, Center for Applied Biotechnology Studies (CABSCon2), December 2017.

These conferences are valuable opportunities for students to learn how to communicate their research and also learn about other research activities by other scientists. The equipment obtained from this grant has helped students collected data so that they can attend more conferences.

Honors and Awards: Nothing to Report

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Technology Transfer: Nothing to Report

PARTICIPANTS:

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Person Months Worked: 10.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

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Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Co-Investigator
Participant: Niroshika Keppetipola
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: K-12 Teacher
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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
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Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

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Participant Type: Undergraduate Student
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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Martin Amezcua
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Ricardo Cruz
Person Months Worked: 9.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Giovanna Cano
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Alejandro Torres
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Elizabeth Morales
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

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Participant Type: Undergraduate Student
Participant: Alejandra Palomino
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Earnest Armenta
Person Months Worked: 10.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Nothing to report in the uploaded pdf